

*W. J. Fry*

AN EXPERIMENTAL ANALYSIS OF THE MICRO-NEUROANATOMY OF THE  
CENTRAL NERVOUS SYSTEM

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1.1 Statement of problem. Application of the quantitative anatomical methods developed under the present contract grant for the determination of neural organization of structures of the central nervous system has been expanded and the emphasis has been placed on studies of the vestibular nuclei in the brain stem of the squirrel monkey. The primary objectives of the research, in terms of developing reliable methods for obtaining reproducible data clearly demonstrating quantitative structural organization in brain systems and the ability to derive the "complete" neuron circuitry by such methods, have been satisfactorily demonstrated in the limbic system of the cat.

The deeper insight into the anatomical relations which the present research has provided indicates definitely that it is desirable to pursue investigations in the time relations obtaining between the complex configurations of the dynamic events characteristic of brain operation as an adjunct to the quantitative elucidation of function. The interruption of information along neural pathways by means of focused ultrasound in combination with stimulation means would be a desirable research direction.

## II Summary of Completed Tasks (4-1-65 through 9-30-65)

2.1 Communications. Several lectures were given to medical or biomedical groups during this period, and papers were given at two annual meetings and one international conference. The list below cites these presentations as well as the title of a paper accepted for publication by the Anatomical Record. Reprints of this article and the paper presented in Tokyo will be forwarded when they are received.

- a. "Mammillary Complex of Cat Brain--Quantitative Organization" at American Association of Anatomists 78th Annual Meeting, Miami Beach, Florida, April, 1965. The Anatomical Record, Vol. 151, #3, 151-2, March 1965 (abstract).
- b. "Quantitative Neuroanatomic Relations in the Mammillary Complex of Cat Brain" at American Academy of Neurology, 17th Annual meeting, Cleveland, Ohio, April, 1965.
- c. "Ultrasound in Biomedical Engineering" at Annual Spring Seminar Series, University of Texas, Austin, Texas, April, 1965.
- d. "Brain Circuitry" at Biomedical Engineering Seminar, Rice University, Houston, Texas, April, 1965.

- e. "Ultrasound as a Research Tool in Biomedical Studies" at Medical Seminar at Bowman-Gray School of Medicine, Winston-Salem, N. C., May, 1965.
- f. "Methods for Completely Deciphering the Neuronal Circuitry of Brain Structures". Digest of the 6th International Conference on Medical Electronics and Biological Engineering, August 22-27, 1965, Tokyo. 278-283 (Invited lecture).
- g. "Mammillary Complex of Cat Brain--Aspects of Quantitative Organization" W. J. Fry. Accepted for publication in the Anatomical Record.

2.2 Preliminary Procedures and Training. Much preliminary work on the vestibular system in the squirrel monkey has been completed. This work entailed the establishment of appropriate surgical, ventriculographic and irradiation procedures for the squirrel monkey. Dosages and position for lesion production have been obtained and monkeys are being irradiated and placed in "storage", i.e., maintained until sufficient time has elapsed for retrograde and transneuronal degeneration in the nuclei of interest to occur.

Three technicians have completed an extensive training period which strengthens the personnel in areas where an increased work load is already evident. These areas are cell identification and counting, cell measurement and histological preparation.

2.3 Instrumentation (mechanical). Although our stereotaxic head holders, associated X-ray equipment and irradiation techniques had been time tested by use on hundreds of cats, a total alignment check of all inter-related elements was undertaken. This is a necessary part of the quality control activity in precision stereotaxic work. The problem is essentially that of providing three orthogonal X-ray axes in space, which are fixed with respect to the animal head holder, along which and parallel to a system of three orthogonal mechanical motions are provided. These mechanical motions are parallel to the X-ray axes to within  $\pm 0.002''$  over the entire working range and it is necessary and possible to bring the zeros of the coordinates of each system into coincidence within  $\pm 0.001''$ . To accomplish this task the machining details must first be precise and there must be precision checking apparatus provided to check the machining accuracy. We use a precision alignment telescope in conjunction with a precision milling machine base as a part of the check out procedure. An X-ray target, consisting of a stable lucite block with lead inserts, has been constructed to provide a rapid and accurate check on X-ray alignment in the future.

Three cast stainless steel head holders were processed in the machine shop to be

interchangeable with positional error of less than  $\pm 0.001$  inch. Histological study of sacrificed animals irradiated with this newly aligned equipment indicated very accurate lesion placement. (See instrumentation, electronic and ultrasonic for additional procedural improvements which also contributed to this successful and accurate lesion generation).

2.4 Instrumentation (Electronic and Ultrasonic). This laboratory developed and uses thermocouple probes for ultrasonic field plotting and calibration of the focused ultrasonic heads used in lesion production. A more thorough check of the mechanical alignment in the calibration procedure has removed the need for a small fixed correction factor.

2.5 Stereotaxia. A sagittal map (see figures 1 and 2) has been completed emphasizing the structure of the brain stem of the squirrel monkey. The Emmers and Ekers stereotaxic atlas of the squirrel monkey was used for site plotting. This map will be used for landmark location in lesion generation and will be updated and/or modified as experience shows necessary after histological examination of the squirrel monkeys sacrificed in our laboratory. In addition, we will be constructing similar brain maps from our sacrificed animals.

2.6 Irradiation. Four dose and position squirrel monkeys have been irradiated in three different sites in the medial longitudinal fasciculus. Results were indicated under 2.3 and 2.4 above. In section 2.2 additional monkeys were said to be put in "storage". Such animals are currently in "storage".

2.7 Anatomy. The boundary outlines of the vestibular nuclei for one squirrel monkey has been essentially completed. This has required the concentrated effort of one professional staff member for about three months. The establishment of criteria for determining these boundaries will materially facilitate future boundary determinations.

### III Work in Progress.

3.1 Instrumentation (Electronic and Data Processing). The automatic readout on the cell measurement microscopes had been converted to punched paper tape using the 8-hole IBM code, and a computer program for automatically processing the data is being checked at present. This will accomplish two worthwhile aims: (1) reduce human transcribing errors and (2) greatly decrease the data processing time. Two microscopes, in closely controlled environmental housings, have received this modification.

SQUIRREL MONKEY

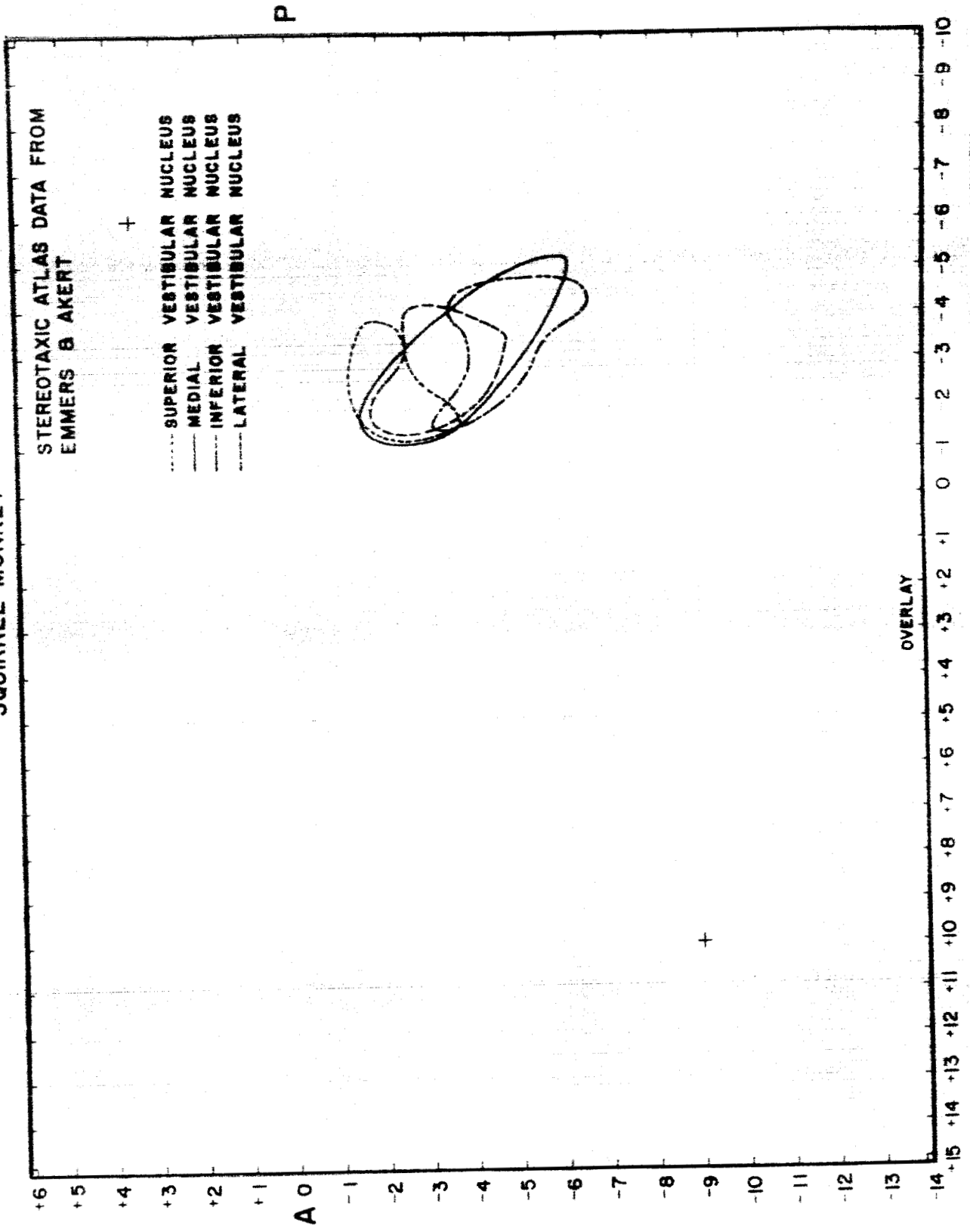


FIGURE I VESTIBULAR NUCLEI PROJECTION ON MID-SAGITTAL PLANE FOR SQUIRREL MONKEY.

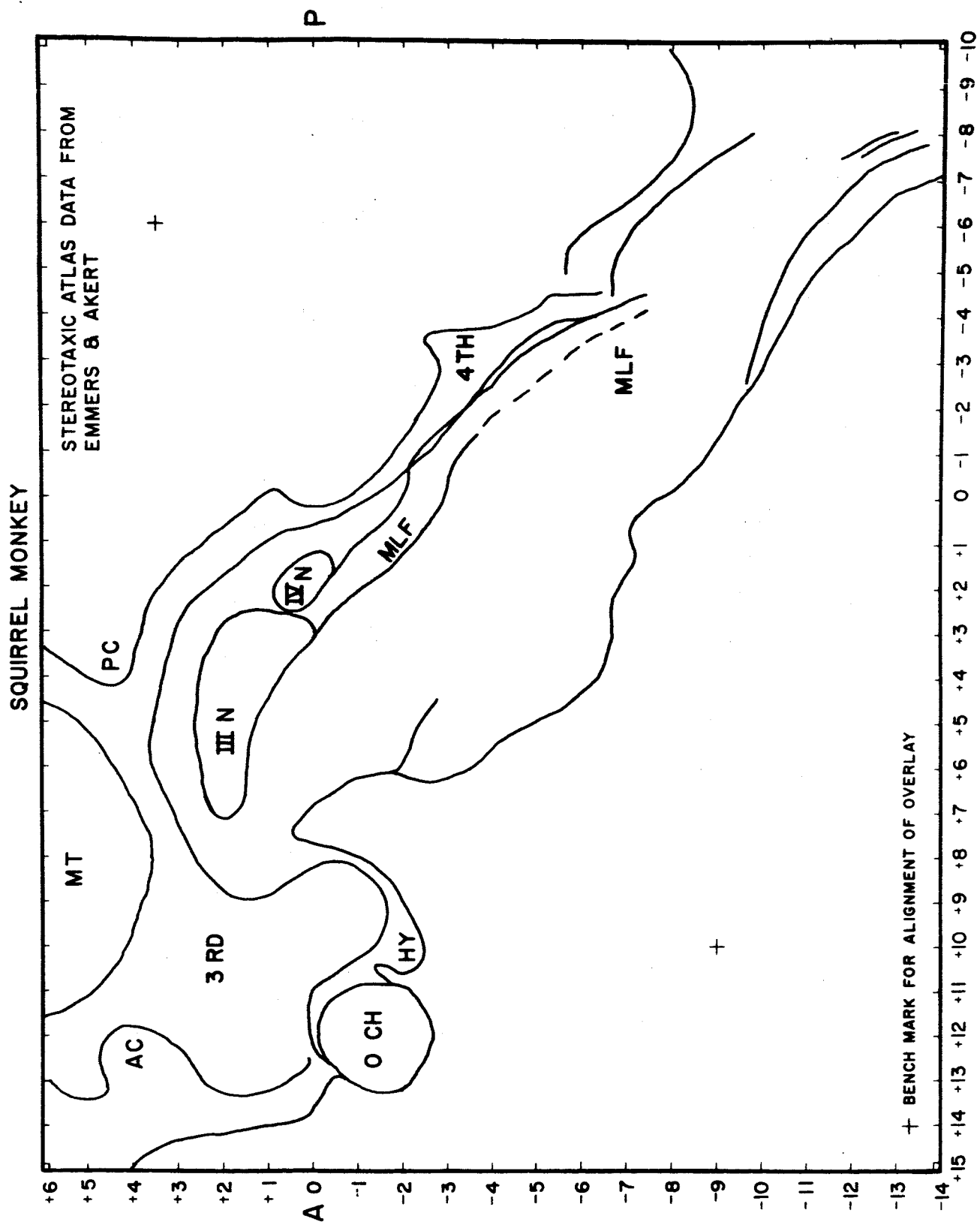


FIGURE II STRUCTURE PROJECTION ON MID-SAGITTAL PLANE FOR BRAIN STEM OF SQUIRREL MONKEY.

Initial consideration is being given to two other problems involved with data acquisition and irradiation. Currently the ventriculographic X-rays are read under a dissecting microscope and an etched glass scale is used to make the measurements necessary to obtain the data required for calculating irradiation positioning coordinates. A mechanical read out, with a binary scale, could be fed to a small computer to expedite the calculations and eliminate human errors.

The other long range major instrumentation advance is the provision for computer controlled automatic irradiation. Such a set up would allow the irradiation of several animals per day instead of the present 2 per day maximum under the most favorable conditions. And once more, the ever present human error would be largely eliminated.

3.2 Cell Counting. Cell counting in "normal" nuclei is proceeding to establish the total "normal" counts which are of interest. Along with this procedure is the necessary cell identification process. It will be some time before any data can be taken on animals with lesion modifications in terms of cell counting because of the wait time for retrograde and transneuronal degeneration processes.

3.3 Irradiation. Animals will be irradiated on regular schedule and placed in "storage" for the degenerative processes to procede.